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(54) **Pharmaceutical preparations containing polyunsaturated fatty acids, their esters or salts, together with antioxidant vitamins or provitamins**

Pharmazeutische Zubereitungen enthaltend mehrfach ungesättigte Fettsäuren zusammen mit antioxydativen Vitaminen und Provitaminen

Compositions pharmaceutiques contenant des acides gras polyinsaturés avec des vitamines et provitamines antioxydantes

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EP-A- 0 295 331 **GB-A- 2 218 904**

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Description

Pharmaceutical preparations are described, useful in the prevention and/or treatment of atherosclerosis, of cardiovascular, nervous system, skin and malignant pathologies, containing 50 to 1000 mg of omega-3 fatty acids, their esters and their salts with inorganic and organic bases, as individual compounds or as a mixture, in combination with 20-30% by weight of antioxidant/reducing vitamins or provitamins.

The present invention refers to pharmaceutical preparations containing omega-3 polyunsaturated fatty acids and suitable quantities of vitamins or provitamins having reducing or antioxidant activity, to be used in the treatment of atherosclerosis or of other pathologies both of the cardiovascular or not cardiovascular type, such as, in example, diseases of the central nervous system, of the skin, inflammatory or neoplastic conditions, etc. The combined use of omega-3 polyunsaturated fatty acids and of highly fatsoluble vitamins, as subsequently described, next to the above mentioned pharmacologic and therapeutic action which will be discussed in due time, also leads to reciprocal potentiation of the respective fat-solubility, and, in the end, to better availability of their combination as compared to the individual constituents. 4,7,10,13,16,19-cis docosahexaenoic acid (hereinafter called DHA) and 5,8,11,14,17-cis eicosapentaenoic acid (hereinafter called EPA) are preferred as omega-3 polyunsaturated fatty acids.

They are used, in conformity with the invention, as such or in the form of esters, preferably ethylesters, or in the form of inorganic salts, for example with alkaline or alkaline-earth metals, or of organic salts, for example with ethanolamine, choline, and alkanolamines in general, alkylamines or finally with basic amino acids, preferably arginine or lysine.

It is understood that DHA and EPA, in any of the above described forms, can be used individually, or as a mixture of the two. The mixtures are prepared by combining the desired quantities of the purified constituents; alternatively the DHA and EPA mixtures or the mixtures of their esters or salts thereof are used, as obtained by extraction, purification and concentration processes of the two acids starting from their natural sources (mainly fish oils).

As regards the reducing vitamins, the vitamins E, A and C are preferably used. The term 'vitamin E' is to be understood as inclusive of α -tocopherol, both in the d- and dl- form, as well as the other tocopherols (β , γ , etc.) in the various stereoisomeric or racemic forms, while the term 'vitamin A' stands for retinal and dihydroretinol. Derivatives of vitamin E and of vitamin A, endowed with vitamin-like action, such as the esters, preferably the acetates, succinates, palmitates, are also conveniently used. In conformity with the invention, the so-called 'provitamins A', that is, the carotenes, preferably β - and α -carotene can also be associated with DHA and EPA. Vitamin C is also conveniently used in combination with EPA and DHA. Owing to its fat-insolubility, this vitamin is used in the form of an ester, specially the palmitate. Alternatively it is used as a sodium salt together with EPA and DHA salts, in aqueous liquid formulations. The reducing vitamins or provitamins are combined with DHA and EPA in substantial quantities, 20% to 30% by weight.

Long chain polyunsaturated fatty acids, in particular those of the omega-3 series, of which DHA and EPA are the most interesting, have recently stimulated great interest as agents useful in the prevention and treatment of numerous pathological forms, specially those related to the cardiocirculatory system. The literature has described DHA and EPA effects against platelet aggregation, as well as their antiatheromatous and antithrombotic action (cf. for instance A. Leaf and P.C. Weber, New England J. Med., 318, 549, 1988) and their antihypertriglyceridemic and antihypercholesterolemic actions. Additional possible instances of DHA and EPA application are the inflammatory pathologies (cf. S.P. Prescott, J. Biol. Chem., 259, 7615, 1984), some neoplastic forms (cf. H.O. Bang et al., Acta Med. Scand., 220, 69, 1976), psoriasis, memory and learning disturbances, some central nervous and peripheral pathologies, etc.

Specially interesting is the antiatherogenous action of EPA and DHA, above all in view of atherosclerosis wide diffusion in the affluent society, in relation also to the alimentary habits and life style. Though thorough studies are still ongoing, it is already possible to advance hypotheses on the mechanism through which this action takes place. The omega-3 fatty acids, by replacing arachidonic acid as cyclo-oxygenase substrates, are oxidised to thromboxane A₃ (not so active as platelet pro-aggregant and vasoconstrictor as thromboxane A₂, an arachidonic acid metabolite) and to prostaglandin PGI₃, having, by converse, anti-aggregant and vasodilator properties similar to those of prostaglandin PGI₂, produced through oxidation of arachidonic acid. The final overall result is a more powerful inhibition of platelet aggregation and an increased vasodilation, that is, a more favourable interaction between platelets and vascular wall.

On the other hand, however, experimental tests *in vitro* and *in vivo* have drawn the attention to a possible cause of troubles in the dietetic or therapeutic use of DHA and EPA or of their derivatives. Their administration, in addition to the other effects, leads to their incorporation into the lipoproteins which, precisely for the increased presence of highly unsaturated acids, become more susceptible to oxidative changes. It is known that oxidation of low-density proteins (LDL) is one of the first stages in the atherogenesis process, stimulating the vascular wall cells to produce cytokines, a cause of local inflammatory reaction (cf. for instance De Maat, Princen, Thromb. Haemost., 69, 6, 1069, 1993). The onset of thrombosis and atherosclerosis is favoured, manifestations which are, paradoxically, two pathologic conditions against which DHA and EPA have shown, in other respects, a marked therapeutic potential.

It has now been found that administration of DHA and EPA, as such or in the form of esters or of salts, in combination with suitable quantities of vitamin E, as previously defined, substantially inhibits the oxidative processes *in vivo* of

the two polyunsaturated acids and for this reason counteracts the oxidation processes, specially those involving LDL, which in the end, favour the onset of atherosclerosis.

Next to the specific action against oxidation of DHA and EPA incorporated in the LDLs, vitamin E carries out an effective action in vivo also against other oxidation and peroxidation processes involved in the genesis of atherosclerosis, i.g., through effective prevention of free-radical (superoxide anion, singlet oxygen, etc.) and hydrogen peroxide formation, now known to favour the onset of atherosclerotic, ischemic and neoplastic diseases.

Also vitamin A and provitamins A (carotenes), as well as vitamin C (ascorbic acid), present in blood, even though in much smaller quantity than that of α -tocopherol, perform an antioxidative action against LDLs, contributing to inhibit atherogenetic processes. The foregoing information defines the advantage of DHA and EPA use in combination with reducing vitamins or provitamins in the treatment and in the prevention of atherosclerosis, as well as in other pathologic conditions of the cardiovascular system connected with atherosclerosis.

In the course of our work on LDL oxidizability we have surprisingly found that the effect on LDL oxidation by EPA and DHA in combination with reducing vitamins and provitamins is not simply additive but produces a true synergistic effect. In a typical experiment, different groups of rats were treated for 6 weeks as follows:

- Group 1: 8 mg/kg of vitamin E (dl- α -tocopherol)
- Group 2: 25 mg/kg of DHA
- Group 3: 8 mg/kg of vitamin E + 25 mg/kg of DHA (in the ratio of about 30:1 00)
- Group 4: 25 mg/kg of an EPA+DHA ethyl ester mixture (total ethyl esters 85%; EPA/DHA ratio 1/1)
- Group 5: 8 mg/kg of vitamin E + 25 mg/kg of the above mentioned mixture of EPA+DHA ethyl esters (in the ratio of about 30:100)
- Group 6: 20 mg/kg of vitamin E + 25 mg/kg of the above mentioned mixture of EPA+DHA ethyl esters (in the ratio of about 80:100)
- Group 7: 1.25 mg/kg of vitamin E + 25 mg/kg of the above mentioned mixture of EPA+DHA ethyl esters (in the ratio of about 5:100)

At the end of treatment the blood LDL fraction was isolated and Oxidised with 5M Cu^{2+} at 37°C for 8 h, the oxidation progress being controlled at the following times: 0, 1, 3, 5, 8 h (cif. Jialal, Vega et al., Atherosclerosis, 84, 185, 1990). To measure oxidation the Thiobarbituric Acid-Reacting Substances (TBARS) test, expressed as malondialdehyde (MDA) equivalent, was used, according to a known method (cif. Jialal, Freeman et al., Arterioscl. Thromb., 11, 482, 1991). The following table I reports the test results.

TABLE 1

LDL oxidation speed, expressed as nanomols of MDA/mg of proteins/hour, after 6 weeks of treatment per os in rats.	
Control group	23.1
Group 1 (vitamin E)	15.5
Group 2 (DHA)	27.3
Group 3 (vit. E 30% with DHA)	9.8
Group 4 (EPA+DHA ethylesters)	27.7
Group 5 (vit. E 30% with EPA + DHA esters)	9.3
Group 6 (vit. E 80% with EPA + DHA esters)	10.1
Group 7 (vit. E 5% with EPA + DHA esters)	21.9

From the above results EPA and DHA, individually (DHA) or in a mixture (EPA+DHA esters), are shown to increase LDL oxidation to a not negligible extent, while the combined use of EPA/DHA plus a quantity equivalent to the 30% of their weight of vitamin E considerably decreases oxidation, to an extent higher than vitamin E alone. The use of a quantity equivalent to 80% of vitamin E together with EPA+DHA ethylesters (group 6) affords no special advantage as compared to the 30% while a limited quantity, in the order of 5% (group 7) has practically no effect.

With reference to the literature on the subject, it is worth mentioning that only German Patent no. 3.719.097 claims the use of EPA and DHA in combination with high quantities of vitamin E, for normalisation of blood coagulation (which undergoes alteration during treatment with EPA and DHA alone), differently from the present patent application.

Said German Patent particularly claims a drug containing EPA and DHA as well as vitamin E, characterized in that the content in vitamin E is 40-100% by weight in respect of the fatty acids and states that smaller quantities have no therapeutic action (they cannot normalise prothrombin time): on the contrary, we have found that, in order to counteract oxidation of the LDLs (and therefore - among others - the onset of atherosclerosis), much smaller quantities of vitamin

are already sufficient to obtain maximum efficacy, ranging such quantities from 20 to 30% of the weight of the fatty acids. Higher quantities have no additional activity and are not considered useful, and might even prove toxic considering the high doses of polyunsaturated acids advocated for clinical use.

Other Authors report the possibility of adding vitamin E (or other antioxidants) for the preservation of polyunsaturated fatty acids *in vitro*. In all these cases however the quantity normally used as an antioxidant *in vitro* is quite small and does not exceed a small percentage by weight in respect to the active substance (1-2% maximum). If at all, British Patent no. 2.218.904 briefly mentions the therapeutic use of DHA ethyl ester combined with 5-10% of α -tocopherol and claims the relative formulation. At these concentrations, however, we have shown that tocopherol effect on *in vivo* peroxidation of the omega-3 acids incorporated in the LDLs is practically negligible (cf. Table 1 - group 7).

On the contrary, the pharmaceutical formulations, subject of the present invention are characterized by substantial quantities of reducing vitamins or provitamins which protect DHA and EPA from oxidation *in vivo* by interacting with the metabolic processes, that is, just as already reported, in the ratio of 20-30% in respect to the polyunsaturated acids.

All in all, it can be concluded that the use of EPA/DHA or of their salts and derivatives in combination with reducing vitamins or provitamins optimize the therapeutic activity of omega-3 polyunsaturated acids, meanwhile inhibiting oxidation of said acids, which is a possible cause of serious side effects such as the onset of atherosclerosis.

Moreover, as already mentioned, the concomitant use of highly fat-soluble substances such as EPA and DHA, and of vitamins E, A, C and carotenes, enhances their reciprocal absorption capacity and their bioavailability following administration by oral route.

Also as regards other types of pathology against which DHA and EPA are used (such as inflammation, psoriasis, neoplastic forms, memory and learning disturbances, central and peripheral nervous system diseases), the concomitant presence of reducing vitamins or provitamins having *in vivo* a protection effect on DHA and EPA is anyhow beneficial.

This effect is quite different and more complex than the already mentioned simple antioxidative effect *in vitro* which the tocopherols, the carotenes and vitamin A and C could have on the oxidizable substances.

The optimal quantity of DHA and EPA acids, their esters or salts, together with the established quantity of reducing vitamin or provitamin may be distributed into soft gelatin capsules, using conventional techniques, solvents and diluents, such as triacetin, polyethyleneglycol mixtures (PEG 200-600 + PEG 4000), the tweens optionally in a mixture with small quantities of ethanol, propylene glycol etc. As an alternative, the acids and the reducing vitamins and provitamins are adsorbed on highly porous material (for example suitably treated silica or alumina) or on materials with which they form true inclusion complexes (e.g., zeolites and cyclodextrins), obtaining solid powders which can be formulated e.g., in the form of tablets, coated tablets etc. If EPA and DHA are used in the form of salts and antioxidant water-soluble vitamins, e.g., vitamin C (ascorbic acid), in the form of sodium salt, the compositions may be in liquid form (drops, syrups, single-dose drinkable vials etc.). Finally, by using suitable excipients and adjuvants, topical dosage forms such as creams may be obtained.

The following examples further illustrate the invention.

Formulation as soft gelatin capsules

Example no. 1	
DHA ethylester (325.6 mg), equivalent to DHA	300 mg
dl- α -tocopherol	90 mg
Triacetin	100 mg
Gelatin	127 mg
Ethyl and propyl p-hydroxybenzoate sodium. respect.	0.58 and 0.29 mg

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DHA ethanolamine salt (339.4 mg), equivalent to DHA	300 mg
dl-(α - tocopherol acetate	65 mg
Triacetin, gelatin, ethyl and propyl p-hydroxybenzoate sodium:	as in Example no. 1

Example no. 3

EPA salt of arginine (454.9 mg) equivalent to EPA	300 mg
Retinol acetate	70 mg
Triacetin, gelatin, ethyl and propyl p-hydroxybenzoate sodium:	as in Example no. 1

Example no. 4

DHA ethylester (162.8 mg) equivalent to DHA	150 mg
EPA ethylester (163.9 mg) equivalent to EPA	150 mg
dl- α -tocopherol	60 mg
Triacetin, gelatin, ethyl and propyl p-hydroxybenzoate sodium :	as in Example no. 1

Example no. 5

EPA	1000 mg
Ascorbyl palmitate	250 mg
Triacetin	160 mg
Gelatin	220 mg
Ethyl and propyl p-hydroxybenzoate sodium	1.9 and 0.97 mg

Formulation as hard gelatin capsules**Example no. 6**

DHA	300 mg
dl- α -tocopherol	59 mg
Ventilated silica	600 mg

(continued)

Example no. 6	
Magnesium stearate	15 mg
Gelatin	125 mg
Ethyl and propyl p-hydroxybenzoate sodium	0.58 and 0.29 mg

Claims

1. Pharmaceutical compositions, useful in the prevention and /or treatment of antherosclerosis as well as cardiovascular, nervous system, skin and malignant diseases, comprising 50 to 1.000 mg of omega-3-fatty acids, their esters and their organic or inorganic salts, as single compounds or as a mixture thereof, in combination with reducing/antioxidant vitamins or provitamins in an amount of 20 to 30% by weight of the fatty acids
2. Pharmaceutical compositions according to claim 1, characterized in that the omega-3- polyunsaturated fatty acids are 4,7,10,13,16,19-cis docosahexaenoic acid (DHA), 5,8,11,14,17-cis eicosapentaenoic acid (EPA), their ethyl esters, salts with alkaline or alkaline-earth bases, ethanolamine, N-methyl and N,N-dimethylalkanolamines, choline, C₁-C₄ alkylamines, arginine, lysine or mixtures thereof.
3. Pharmaceutical compositions according to claim 1, characterized in that the antioxidant vitamins are vitamin E (tocopherol α , β , γ , etc.) in any stereoisomeric or racemic form, vitamin A (retinol or dihydroretinol) or their esters, vitamin C or its sodium salt or palmitate ester and that the antioxidant provitamins are α -, β - and γ -carotene.

Patentansprüche

1. Pharmazeutische Zusammensetzungen zur Vorbeugung und/oder Behandlung von Arteriosklerose und kardiovaskulären, Nervensystem-, Haut- und Tumor-Krankheiten umfassend 50 bis 1000 mg von omega-3-Fettsäuren, deren Estern und deren organischen oder anorganischen Salzen als einzelne Verbindungen oder als ein Gemisch davon in Verbindung mit reduzierende/antioxidierenden Vitamins oder Provitamins in einer Menge von 20 bis 30 Gew.-% der Fettsäuren.
2. Pharmazeutische Zusammensetzungen nach Anspruch 1, dadurch gekennzeichnet, daß die omega-3-mehrfach- ungesättigten Fettsäuren sind 4, 7, 10, 13, 16, 19-cis-Docosahexaensäure (DHA), 5, 8, 11, 14, 17-cis-Eikosapentaensäure (EPA), deren Ethylester, Salze mit basischen Alkali- oder Erdalkalimetallbindungen, Ethanolaminen, N-Methyl- und N, N-Dimethylalkanolaminen, Cholin, C₁-C₄-Alkylaminen, Arginin, Lysin oder Gemische davon.
3. Pharmazeutische Zusammensetzungen nach Anspruch 1, dadurch gekennzeichnet, daß die antioxidierenden Vitamine Vitamin E (Tocopherol, α , β , γ , etc.) in irgendeiner stereoisomeren oder racemischen Form, Vitamin A (Retinol oder Dihydroretinol) oder deren Ester, Vitamin C oder dessen Natriumsalz oder Palmitatester sind, und daß die antioxidierenden Provitamine α -, β -, γ -Carotine sind.

Revendications

1. Compositions pharmaceutiques utiles dans la prévention et/ou le traitement d'athérosclérose ainsi que de maladies cardiovasculaires, du système nerveux, de la peau et malignes, comprenant 50 à 1.000 mg d'acides gras oméga-3, de leurs esters et de leurs sels organiques ou inorganiques, sous forme de composés uniques ou d'un mélange de ceux-ci, en combinaison avec des vitamines ou provitamines réductrices/antioxydantes en une quantité de 20 à 30% en poids des acides gras.
2. Compositions pharmaceutiques suivant la revendication 1, caractérisées en ce que les acides gras oméga-3-poly-insaturés sont l'acide 4,7,10,13,16,19-cis-docosahexaénoïque (DHA), l'acide 5,8,11,14,17-cis-éicosapentaénoïque (EPA), leurs esters éthyliques, leurs sels formés avec des bases de métal alcalin ou alcalino-terreux, l'éthanolamine, les N-méthyl- et N,N-diméthyl-alcanolamines, la choline, des alkylamines en C₁₋₄, l'arginine, la lysine ou leurs mélanges.
3. Compositions pharmaceutiques suivant la revendication 1, caractérisées en ce que les vitamines antioxydantes sont la vitamine E (tocophérol α , β , γ , etc.) sous une quelconque forme stéréoisomère ou racémique, la vitamine A

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(rétinol ou dihydrorétinol) ou leurs esters, la vitamine C ou son sel de sodium ou son ester palmitate, et que les pro-vitamines antioxydantes sont l' α , β et γ -carotène.

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